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FOR

“OVOMUCOID PROMOTER AND METHODS OF USE”

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Title of the Invention

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OVOMUCOID PROMOTER AND METHODS OF USE

Field of the Invention

10 The present invention relates generally to the identification of an avian ovomucoid gene expression control region. More specifically, the invention relates to recombinant nucleic acids and expression vectors, transfected cells and transgenic animals, especially chickens, that comprise the avian ovomucoid gene expression control region operably linked to a polypeptide-encoding nucleic acid.

15

Background

20 The field of transgenics was initially developed to understand the action of a single gene in the context of the whole animal and the phenomena of gene activation, expression, and interaction. This technology has also been used to produce models for various diseases in humans and other animals and is amongst the most powerful tools available for the study of genetics, and the understanding of genetic mechanisms and function. From an economic perspective, the use of transgenic technology to convert animals into "protein factories" for the production of specific proteins or other substances of pharmaceutical interest (Gordon et al., 1987, *Biotechnology* 5: 1183-1187; Wilmot et al., 1990, *Theriogenology* 33: 113-123) offers significant advantages over more conventional methods of protein production by gene expression.

25

Heterologous nucleic acids have been engineered so that an expressed protein may be joined to a protein or peptide that will allow secretion of the transgenic expression product into milk or urine, from which the protein may then be recovered. These procedures have had limited success and may require lactating animals, with the attendant costs of maintaining individual animals or herds of large species, including cows, sheep, or goats.

Historically, transgenic animals have been produced almost exclusively by microinjection of the fertilized egg. The pronuclei of fertilized eggs are microinjected *in vitro* with foreign, *i.e.*, xenogeneic or allogeneic, heterologous DNA or hybrid DNA molecules. The microinjected fertilized eggs are then transferred to the genital tract of a pseudopregnant female (e.g., Krimpenfort et al., in U.S. Pat. No. 5,175,384).

One system that holds potential is the avian reproductive system. The production of an avian egg begins with formation of a large yolk in the ovary of the hen. The unfertilized oocyte or ovum is positioned on top of the yolk sac. After ovulation, the ovum passes into the infundibulum of the oviduct where it is fertilized, if sperm are present, and then moves into the magnum of the oviduct lined with tubular gland cells. These cells secrete the egg-white proteins, including ovalbumin, ovomucoid, lysozyme, conalbumin and ovomucin, into the lumen of the magnum where they are deposited onto the avian embryo and yolk.

The hen oviduct offers outstanding potential as a protein bioreactor because of the high levels of protein production, the promise of proper folding and post-translation modification of the target protein, the ease of product recovery, and the shorter developmental period of chickens compared to other potential animal species. As a result, efforts have been made to create transgenic chickens expressing heterologous proteins in the oviduct by means of microinjection of DNA (PCT Publication WO 97/47739).

Chicken oviduct cells, when stimulated by steroid hormones during egg-laying, secrete three principal polypeptides, ovalbumin, ovomucoid and lysozyme (Tsai et al., 1978, *Biochemistry* 17: 5773-5779). The mRNA transcript encoding ovalbumin constitutes about 50% of the total mRNA of these cells. Ovomucoid and lysozyme mRNAs contribute about 6.6% and 3.4% respectively of the total mRNA of the steroid stimulated cells (Hynes et al., 1977, *Cell* 11:923-932).

Detailed restriction enzyme analysis of fragments of chicken genomic DNA have shown that the ovomucoid-encoding sequence includes seven intronic sequences (Lindenmaier et al., 1979, *Nuc. Acid Res.* 7:1221-1232; Catterall et al., 1979, *Nature* 278: 323-327; Lai et al., 1979, *Cell* 18:829-842). Short stretches of the 5' flanking region of the ovomucoid gene have been sequenced (Lai et al., 1979, *Cell* 18: 829-842; Genbank Accession No. J00897), but extending only 579 bases upstream of the recognized transcription start site. The 5' flanking region of the ovomucoid gene has been isolated (Catterall et al., 1979, *Nature* 278: 323-327); Lai et al., 1979, *Cell* 18: 829-842) but not generally characterized beyond low-resolution restriction site mapping. Scott et al. identified a CR1-like region within the 10 kb chicken genomic DNA located between the ovoinhibitor-encoding region and the downstream ovomucoid gene (1987, *Biochemistry* 26: 6831-6840). The ovoinhibitor-encoding cDNA and the attached 3'-untranslated region, which extends into the 10 kb ovoinhibitor-ovomucoid region, were also sequenced (Scott et al., 1987, *J. Biol. Chem.* 262: 5899-5907).

The chicken ovomucoid gene, therefore, is highly expressed in the tubular glands of the mature hen oviduct and represents a suitable candidate for an efficient promoter for heterologous protein production in transgenic animals, especially chickens. The regulatory region of the ovomucoid locus extends over a nucleic acid region of at about 10 kb of DNA 5' upstream of the transcription start site, and comprises at least one recognized element, the CR1.

Summary of the Invention

Briefly described, the present invention relates to a novel isolated avian nucleic acid comprising an avian ovomucoid gene expression control region.

5 The isolated nucleic acid of the present invention is useful for the expression of an operably linked heterologous nucleic acid insert in a transfected avian cell such as, for example, an oviduct cell.

One aspect of the present invention provides a novel isolated nucleic acid that is located immediately 5' upstream of a transcription start site of the
10 chicken ovomucoid gene locus. The novel isolated avian nucleic acid sequence encoding an ovomucoid gene expression control region comprises at least one avian CR1 repeat element, and a proximal ovomucoid promoter. Interspersed between these constituent elements are stretches of nucleic acid that may serve at least to organize the gene regulatory elements in an
15 ordered array relative to a polypeptide-encoding region. In one embodiment of the present invention the isolated nucleic acid is isolated from a chicken.

The isolated avian ovomucoid promoter region of the present invention is useful for directing expression of a polypeptide-encoding nucleic acid which, in one embodiment, is tissue specific expression. The isolated avian
20 ovomucoid promoter may be operably linked with a selected nucleic acid insert, wherein the nucleic acid insert encodes a polypeptide desired to be expressed in a transfected cell. The nucleic acid insert may be placed in frame with a signal peptide sequence. Translation initiation may start with the signal peptide and continue through the nucleic acid insert, thereby producing
25 an expressed polypeptide having the desired amino acid sequence.

The recombinant DNA of the present invention may further comprise a polyadenylation signal sequence that will allow the transcript directed by the novel ovomucoid gene expression control region to proceed beyond the nucleic acid insert encoding a polypeptide and allow the transcript to further
30 comprise a 3' untranslated region and a polyadenylated tail. Any functional

polyadenylation signal sequence, including but not limited to the SV40 polyadenylation signal sequence, bovine growth hormone adenylation sequence or the like, may be linked to the 3' end of the nucleic acid insert.

5 The sequence of the expressed nucleic acid insert may be optimized for codon usage by a host cell. This may be determined from the codon usage of at least one, and preferably more than one, protein expressed in a chicken cell. For example, the codon usage may be determined from the nucleic acid sequences encoding the proteins ovalbumin, ovomucoid, ovomucin, ovotransferrin, and the like in a chicken.

10 Yet another aspect of the present invention is directed to an expression vector suitable for delivery to a recipient cell for expression of the vector therein. The expression vector of the present invention may comprise an isolated avian ovomucoid gene expression control region operably linked to a nucleic acid insert encoding a polypeptide and, optionally, a polyadenylation signal sequence. The expression vector may further
15 comprise a bacterial plasmid sequence, a viral nucleic acid sequence, or fragments or variants thereof that may allow for replication of the vector in a suitable host.

Another aspect of the present invention is a method of expressing a
20 heterologous polypeptide in a eukaryotic cell by transfecting the cell with a recombinant DNA comprising an avian ovomucoid gene expression control region operably linked to a nucleic acid insert encoding a polypeptide and, optionally, a polyadenylation signal sequence, and culturing the transfected cell in a medium suitable for expression of the heterologous polypeptide
25 under the control of the avian ovomucoid gene expression control region.

Also within the scope of the present invention are recombinant cells, tissues, and animals containing non-naturally occurring recombinant nucleic acid molecules according to the present invention and described above. In one embodiment of the present invention, the transformed cell is a chicken
30 oviduct cell and the nucleic acid insert comprises the chicken ovomucoid

gene expression control region, a nucleic acid insert encoding a human interferon $\alpha 2d$ and codon optimized for expression in an avian cell, and an SV40 polyadenylation sequence.

Additional objects and aspects of the present invention will become
5 more apparent upon review of the detailed description set forth below when
taken in conjunction with the accompanying figures, which are briefly
described as follows.

Brief Description of the Figures

10 Fig. 1 illustrates an agarose gel analysis of PCR products from PCR
amplification of chicken genomic DNA using the primers OVINs2 (SEQ ID
NO: 1) and OVMUa2 (SEQ ID NO: 2).

Fig. 2 illustrates the approximately 10 kb nucleic acid region that is 5'
15 upstream of the chicken ovomucoid transcription start site, and the positions
and orientations of primers used to sequence this region.

Fig. 3 shows PCR primers SEQ ID NOS: 1 – 25 used to PCR amplify and/or
sequence the approximately 10 kb nucleic acid region that is 5' upstream of
20 the chicken ovomucoid transcription start site.

Fig. 4 shows the nucleic acid sequence SEQ ID NO: 26 of the approximately
10 kb nucleic acid region that is 5' upstream of the chicken ovomucoid
transcription start site.

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Detailed Description of the Preferred Embodiments

Reference now will be made in detail to the presently preferred
embodiments of the invention, one or more examples of which are illustrated
in the accompanying drawings. Each example is provided by way of
30 explanation of the invention, not limitation of the invention. In fact, it will be

apparent to those skilled in the art that various modifications, combinations, additions, deletions and variations can be made in the present invention without departing from the scope or spirit of the invention. For instance, features illustrated or described as part of one embodiment can be used in
5 another embodiment to yield a still further embodiment. It is intended that the present invention covers such modifications, combinations, additions, deletions and variations as come within the scope of the appended claims and their equivalents.

This description uses gene nomenclature accepted by the Cucurbit
10 Genetics Cooperative as it appears in the *Cucurbit Genetics Cooperative Report* 18:85 (1995), herein incorporated by reference in its entirety. Using this gene nomenclature, genes are symbolized by italicized Roman letters. If a mutant gene is recessive to the normal type, then the symbol and name of the mutant gene appear in italicized lower case letters.

15 For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

Definitions

The term "animal" is used herein to include all vertebrate animals,
20 including humans. It also includes an individual animal in all stages of development, including embryonic and fetal stages.

The term "avian" as used herein refers to any species, subspecies or race of organism of the taxonomic class *ava*, such as, but not limited to, such organisms as chicken, turkey, duck, goose, quail, pheasants, parrots, finches,
25 hawks, crows and ratites including ostrich, emu and cassowary. The term includes the various known strains of *Gallus gallus*, or chickens, (for example, White Leghorn, Brown Leghorn, Barred-Rock, Sussex, New Hampshire, Rhode Island, Ausstralorp, Minorca, Amrox, California Gray, Italian Partidge-colored), as well as strains of turkeys, pheasants, quails, duck, ostriches and
30 other poultry commonly bred in commercial quantities.

The term "nucleic acid" as used herein refers to any natural and synthetic linear and sequential arrays of nucleotides and nucleosides, for example cDNA, genomic DNA, mRNA, tRNA, oligonucleotides, oligonucleosides and derivatives thereof. For ease of discussion, such nucleic acids may be collectively referred to herein as "constructs," "plasmids," or "vectors." Representative examples of the nucleic acids of the present invention include bacterial plasmid vectors including expression, cloning, cosmid and transformation vectors such as, but not limited to, pBR322, animal viral vectors such as, but not limited to, modified adenovirus, influenza virus, polio virus, pox virus, retrovirus, and the like, vectors derived from bacteriophage nucleic acid, and synthetic oligonucleotides like chemically synthesized DNA or RNA. The term "nucleic acid" further includes modified or derivatised nucleotides and nucleosides such as, but not limited to, halogenated nucleotides such as, but not only, 5-bromouracil, and derivatised nucleotides such as biotin-labeled nucleotides.

The term "isolated nucleic acid" as used herein refers to a nucleic acid with a structure (a) not identical to that of any naturally occurring nucleic acid or (b) not identical to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes, and includes DNA, RNA, or derivatives or variants thereof. The term covers, for example, (a) a DNA which has the sequence of part of a naturally occurring genomic molecule but is not flanked by at least one of the coding sequences that flank that part of the molecule in the genome of the species in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic nucleic acid of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any vector or naturally occurring genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), ligase chain reaction (LCR) or chemical synthesis, or a restriction fragment; (d) a recombinant nucleotide sequence that is part of a hybrid gene, *i.e.*, a gene encoding a fusion protein,

and (e) a recombinant nucleotide sequence that is part of a hybrid sequence that is not naturally occurring. Isolated nucleic acid molecules of the present invention can include, for example, natural allelic variants as well as nucleic acid molecules modified by nucleotide deletions, insertions, inversions, or
5 substitutions such that the resulting nucleic acid molecule still essentially encodes an ovomucoid gene expression control region or a variant thereof of the present invention.

By the use of the term "enriched" in reference to nucleic acid it is meant that the specific DNA or RNA sequence constitutes a significantly
10 higher fraction of the total DNA or RNA present in the cells or solution of interest than in normal or diseased cells or in the cells from which the sequence was taken. Enriched does not imply that there are no other DNA or RNA sequences present, just that the relative amount of the sequence of interest has been significantly increased. The other DNA may, for example,
15 be derived from a yeast or bacterial genome, or a cloning vector, such as a plasmid or a viral vector. The term "significant" as used herein is used to indicate that the level of increase is useful to the person making such an increase.

It is advantageous for some purposes that a nucleotide sequence is in
20 purified form. The term "purified" in reference to nucleic acid represents that the sequence has increased purity relative to the natural environment.

The terms "polynucleotide," "oligonucleotide," and "nucleic acid sequence" are used interchangeably herein and include, but are not limited to, coding sequences (polynucleotide(s) or nucleic acid sequence(s) which
25 are transcribed and translated into polypeptide *in vitro* or *in vivo* when placed under the control of appropriate regulatory or control sequences); control sequences (e.g., translational start and stop codons, promoter sequences, ribosome binding sites, polyadenylation signals, transcription factor binding sites, transcription termination sequences, upstream and downstream
30 regulatory domains, enhancers, silencers, and the like); and regulatory

sequences (DNA sequences to which a transcription factor(s) binds and alters the activity of a gene's promoter either positively (induction) or negatively (repression)). No limitation as to length or to synthetic origin are suggested by the terms described herein.

5 As used herein the terms "polypeptide" and "protein" refer to a polymer of amino acids of three or more amino acids in a serial array, linked through peptide bonds. The term "polypeptide" includes proteins, protein fragments, protein analogues, oligopeptides and the like. The term "polypeptides" contemplates polypeptides as defined above that are encoded by nucleic
10 acids, produced through recombinant technology (isolated from an appropriate source such as a bird), or synthesized. The term "polypeptides" further contemplates polypeptides as defined above that include chemically modified amino acids or amino acids covalently or noncovalently linked to labeling ligands.

15 The term "fragment" as used herein to refer to a nucleic acid (e.g., cDNA) refers to an isolated portion of the subject nucleic acid constructed artificially (e.g., by chemical synthesis) or by cleaving a natural product into multiple pieces, using restriction endonucleases or mechanical shearing, or a portion of a nucleic acid synthesized by PCR, DNA polymerase or any other
20 polymerizing technique well known in the art, or expressed in a host cell by recombinant nucleic acid technology well known to one of skill in the art. The term "fragment" as used herein may also refer to an isolated portion of a polypeptide, wherein the portion of the polypeptide is cleaved from a naturally occurring polypeptide by proteolytic cleavage by at least one protease, or is a
25 portion of the naturally occurring polypeptide synthesized by chemical methods well known to one of skill in the art.

 The term "gene" or "genes" as used herein refers to nucleic acid sequences (including both RNA or DNA) that encode genetic information for the synthesis of a whole RNA, a whole protein, or any portion of such whole
30 RNA or whole protein. Genes that are not naturally part of a particular

organism's genome are referred to as "foreign genes," "heterologous genes" or "exogenous genes" and genes that are naturally a part of a particular organism's genome are referred to as "endogenous genes". The term "gene product" refers to RNAs or proteins that are encoded by the gene. "Foreign gene products" are RNA or proteins encoded by "foreign genes" and "endogenous gene products" are RNA or proteins encoded by endogenous genes. "Heterologous gene products" are RNAs or proteins encoded by "foreign, heterologous or exogenous genes" and are, therefore, not naturally expressed in the cell.

The term "expressed" or "expression" as used herein refers to the transcription from a gene to give an RNA nucleic acid molecule at least complementary in part to a region of one of the two nucleic acid strands of the gene. The term "expressed" or "expression" as used herein also refers to the translation from said RNA nucleic acid molecule to give a protein, a polypeptide, or a portion or fragment thereof.

As used herein, the term "locus" or "loci" refers to the site of a gene on a chromosome. Pairs of genes control hereditary traits, each in the same position on a pair of chromosomes. These gene pairs, or alleles, may both be dominant or both be recessive in expression of that trait. In either case, the individual is said to be homozygous for the trait controlled by that gene pair. If the gene pair (alleles) consists of one dominant and one recessive trait, the individual is heterozygous for the trait controlled by the gene pair. Natural variation in genes or nucleic acid molecules caused by, for example, recombination events or resulting from mutation, gives rise to allelic variants with similar, but not identical, nucleotide sequences. Such allelic variants typically encode proteins with similar activity to that of the protein encoded by the gene to which they are compared, because natural selection typically selects against variations that alter function. Allelic variants can also comprise alterations in the untranslated regions of the gene as, for example, in the 3' or 5' untranslated regions or can involve alternate splicing of a

nascent transcript, resulting in alternative exons being positioned adjacently.

The term "operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Control sequences operably linked to a coding sequence are
5 capable of effecting the expression of the coding sequence. The control sequences need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be
10 considered "operably linked" to the coding sequence.

The terms "transcription regulatory sequences" and "gene expression control regions" as used herein refer to nucleotide sequences that are associated with a gene nucleic acid sequence and which regulate the transcriptional expression of the gene. Exemplary transcription regulatory
15 sequences include enhancer elements, hormone response elements, steroid response elements, negative regulatory elements, and the like. The "transcription regulatory sequences" may be isolated and incorporated into a vector nucleic acid to enable regulated transcription in appropriate cells of portions of the vector DNA. The "transcription regulatory sequence" may
20 precede, but is not limited to, the region of a nucleic acid sequence that is in the region 5' of the end of a protein coding sequence that may be transcribed into mRNA. Transcriptional regulatory sequences may also be located within a protein coding region, in regions of a gene that are identified as "intron" regions, or may be in regions of nucleic acid sequence that are in the region
25 of nucleic acid.

The term "promoter" as used herein refers to the DNA sequence that determines the site of transcription initiation from an RNA polymerase. A "promoter-proximal element" may be a regulatory sequence within about 200 base pairs of the transcription start site.

30 The term "coding region" as used herein refers to a continuous linear

arrangement of nucleotides that may be translated into a protein. A full length coding region is translated into a full length protein; that is, a complete protein as would be translated in its natural state absent any post-translational modifications. A full length coding region may also include any leader protein
5 sequence or any other region of the protein that may be excised naturally from the translated protein.

The term "complementary" as used herein refers to two nucleic acid molecules that can form specific interactions with one another. In the specific interactions, an adenine base within one strand of a nucleic acid can form two
10 hydrogen bonds with thymine within a second nucleic acid strand when the two nucleic acid strands are in opposing polarities. Also in the specific interactions, a guanine base within one strand of a nucleic acid can form three hydrogen bonds with cytosine within a second nucleic acid strand when the two nucleic acid strands are in opposing polarities. Complementary
15 nucleic acids as referred to herein, may further comprise modified bases wherein a modified adenine may form hydrogen bonds with a thymine or modified thymine, and a modified cytosine may form hydrogen bonds with a guanine or a modified guanine.

The term "probe" as used herein, when referring to a nucleic acid,
20 refers to a nucleotide sequence that can be used to hybridize with and thereby identify the presence of a complementary sequence, or a complementary sequence differing from the probe sequence but not to a degree that prevents hybridization under the hybridization stringency conditions used. The probe may be modified with labels such as, but not
25 only, radioactive groups, chemiluminescent moieties, biotin, and the like that are well known in the art.

The term "capable of hybridizing under stringent conditions" as used herein refers to annealing a first nucleic acid to a second nucleic acid under stringent conditions as defined below. Stringent hybridization conditions
30 typically permit the hybridization of nucleic acid molecules having at least

70% nucleic acid sequence identity with the nucleic acid molecule being used as a probe in the hybridization reaction. For example, the first nucleic acid may be a test sample or probe, and the second nucleic acid may be the sense or antisense strand of an ovomucoid gene expression control region or
5 a fragment thereof. Hybridization of the first and second nucleic acids may be conducted under stringent conditions, e.g., high temperature and/or low salt content that tend to disfavor hybridization of dissimilar nucleotide sequences. Alternatively, hybridization of the first and second nucleic acid may be conducted under reduced stringency conditions, e.g. low temperature and/or
10 high salt content that tend to favor hybridization of dissimilar nucleotide sequences. Low stringency hybridization conditions may be followed by high stringency conditions or intermediate medium stringency conditions to increase the selectivity of the binding of the first and second nucleic acids. The hybridization conditions may further include reagents such as, but not
15 limited to, dimethyl sulfoxide (DMSO) or formamide to disfavor still further the hybridization of dissimilar nucleotide sequences. A suitable hybridization protocol may, for example, involve hybridization in 6X SSC (wherein 1X SSC comprises 0.015 M sodium citrate and 0.15 M sodium chloride), at 65° C in an aqueous solution, followed by washing with 1X SSC at 65° C. Formulae to
20 calculate appropriate hybridization and wash conditions to achieve hybridization permitting 30% or less mismatch between two nucleic acid molecules are disclosed, for example, in Meinkoth et al., 1984, *Anal. Biochem.* 138: 267-284; the contents of which is incorporated herein by reference in its entirety. Protocols for hybridization techniques are well known
25 to those of skill in the art and standard molecular biology manuals may be consulted to select a suitable hybridization protocol without undue experimentation. See, for example, Sambrook et al., 1989, "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Press: the contents of which is incorporated herein by reference in its entirety.

30 Typically, stringent conditions will be those in which the salt

concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) from about pH 7.0 to about pH 8.3 and the temperature is at least about 30° C for short probes (e.g., 10 to 50 nucleotides) and at least about 60° C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37° Celsius, and a wash in 1x to 2x SSC at 50 to 55° Celsius. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37° Celsius, and a wash in 0.5x to 1x SSC at 55 to 60° Celsius. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37° Celsius, and a wash in 0.1x SSC at 60 to 65° Celsius.

The terms "unique nucleic acid region" and "unique protein (polypeptide) region" as used herein refer to sequences present in a nucleic acid or protein (polypeptide) respectively that is not present in any other nucleic acid or protein sequence. The terms "conserved nucleic acid region" as referred to herein is a nucleotide sequence present in two or more nucleic acid sequences, to which a particular nucleic acid sequence can hybridize under low, medium or high stringency conditions. The greater the degree of conservation between the conserved regions of two or more nucleic acid sequences, the higher the hybridization stringency that will allow hybridization between the conserved region and a particular nucleic acid sequence.

The terms "percent sequence identity" or "percent sequence similarity" as used herein refer to the degree of sequence identity between two nucleic acid sequences or two amino acid sequences as determined using the algorithm of Karlin and Attschul, 1990, *Proc. Natl. Acad. Sci.* 87: 2264-2268, modified as in Karlin and Attschul, 1993, *Proc. Natl. Acad. Sci.* 90: 5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Attschul et al., 1990, *T. Mol. Biol.* Q15: 403-410. BLAST

nucleotide searches are performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleic acid molecule of the invention. BLAST protein searches are performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a reference polypeptide. To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Attschul et al., 1997, *Nucl. Acids Res.* 25: 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g. XBLAST and NBLAST) are used. See
10 <http://www.ncbi.nlm.nih.gov>. Other algorithms, programs and default settings may also be suitable such as, but not only, the GCG-Sequence Analysis Package of the U.K. Human Genome Mapping Project Resource Centre that includes programs for nucleotide or amino acid sequence comparisons.

The term "sense strand" as used herein refers to a single stranded
15 DNA molecule from a genomic DNA that may be transcribed into RNA and translated into the natural polypeptide product of the gene. The term "antisense strand" as used herein refers to the single strand DNA molecule of a genomic DNA that is complementary with the sense strand of the gene.

The term "antisense DNA" as used herein refers to a gene sequence
20 DNA that has a nucleotide sequence complementary to the "sense strand" of a gene when read in reverse orientation, i.e., DNA read into RNA in a 3' to 5' direction rather than in the 5' to 3' direction. The term "antisense RNA" is used to mean an RNA nucleotide sequence (for example, that encoded by an antisense DNA or synthesized complementary with the antisense DNA).
25 Antisense RNA is capable of hybridizing under stringent conditions with an antisense DNA. The antisense RNA of the invention is useful for regulating expression of a "target gene" either at the transcriptional or translational level. For example, transcription of the subject nucleic acids may produce antisense transcripts that are capable of inhibiting transcription by inhibiting initiation of
30 transcription or by competing for limiting transcription factors; the antisense

transcripts may inhibit transport of the "target RNA", or, the antisense transcripts may inhibit translation of "target RNA".

The term "nucleic acid vector" as used herein refers to a natural or synthetic single or double stranded plasmid or viral nucleic acid molecule that can be transfected or transformed into cells and replicate independently of, or within, the host cell genome. A circular double stranded plasmid can be linearized by treatment with an appropriate restriction enzyme based on the nucleotide sequence of the plasmid vector. A nucleic acid can be inserted into a vector by cutting the vector with restriction enzymes and ligating the pieces together. The nucleic acid molecule can be RNA or DNA.

The term "expression vector" as used herein refers to a nucleic acid vector that comprises the ovomucoid gene expression control region operably linked to a nucleotide sequence coding at least one polypeptide. As used herein, the term "regulatory sequences" includes promoters, enhancers, and other elements that may control gene expression. Standard molecular biology textbooks (for example, Sambrook et al., eds., 1989, "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Press) may be consulted to design suitable expression vectors that may further include an origin of replication and selectable gene markers. It should be recognized, however, that the choice of a suitable expression vector and the combination of functional elements therein depends upon multiple factors including the choice of the host cell to be transformed and/or the type of protein to be expressed.

The terms "transformation" and "transfection" as used herein refer to the process of inserting a nucleic acid into a host. Many techniques are well known to those skilled in the art to facilitate transformation or transfection of a nucleic acid into a prokaryotic or eukaryotic organism. These methods involve a variety of techniques, such as treating the cells with high concentrations of salt such as, but not only, a calcium or magnesium salt, an

electric field, detergent, or liposome mediated transfection, to render the host cell competent for the uptake of the nucleic acid molecules, and by such methods as sperm-mediated and restriction-mediated integration.

The term "transfecting agent" as used herein refers to a composition of matter added to the genetic material for enhancing the uptake of heterologous DNA segment(s) into a eukaryotic cell including, but not limited to, an avian cell like a chicken male germ cell. The enhancement is measured relative to the uptake in the absence of the transfecting agent. Examples of transfecting agents include adenovirus-transferrin-polylysine-DNA complexes. These complexes generally augment the uptake of DNA into the cell and reduce its breakdown during its passage through the cytoplasm to the nucleus of the cell. These complexes can be targeted to the male germ cells using specific ligands that are recognized by receptors on the cell surface of the germ cell, such as the c-kit ligand or modifications thereof.

Other preferred transfecting agents include, but are not limited to, lipofectin, lipfectamine, DIMRIE C, Supeffect, and Effectin (Qiagen), unifactin, maxifactin, DOTMA, DOGS (Transfectam; dioctadecylamidoglycylspermine), DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), DOTAP (1,2-dioleoyl-3-trimethylammonium propane), DDAB (dimethyl dioctadecylammonium bromide), DHDEAB (N,N-di-n-hexadecyl-N,N-dihydroxyethyl ammonium bromide), HDEAB (N-n-hexadecylN,N-dihydroxyethylammonium bromide), polybrene, poly(ethylenimine) (PEI) and the like. These non-viral agents have the advantage that they can facilitate stable integration of xenogeneic DNA sequences into the vertebrate genome, without size restrictions commonly associated with virus-derived transfecting agents.

The term "recombinant cell" refers to a cell that has a new combination of nucleic acid segments that are not covalently linked to each other in nature. A new combination of nucleic acid segments can be introduced into an organism using a wide array of nucleic acid manipulation techniques available

to those skilled in the art. A recombinant cell can be a single eukaryotic cell, or a single prokaryotic cell, or a mammalian cell. The recombinant cell may harbor a vector that is extragenomic. An extragenomic nucleic acid vector does not insert into the cell's genome. A recombinant cell may further harbor
5 a vector or a portion thereof that is intragenomic. The term intragenomic defines a nucleic acid construct incorporated within the recombinant cell's genome.

The terms "recombinant nucleic acid" and "recombinant DNA" as used herein refer to combinations of at least two nucleic acid sequences that are
10 not naturally found in a eukaryotic or prokaryotic cell. The nucleic acid sequences include, but are not limited to, nucleic acid vectors, gene expression regulatory elements, origins of replication, suitable gene sequences that when expressed confer antibiotic resistance, protein-encoding sequences, and the like. The term "recombinant polypeptide" is meant to
15 include a polypeptide produced by recombinant DNA techniques such that it is distinct from a naturally occurring polypeptide either in its location, purity or structure. Generally, such a recombinant polypeptide will be present in a cell in an amount different from that normally observed in nature.

As used herein, a "transgenic animal" is any animal, such as an avian
20 animal including a chicken, in which one or more of the cells of the animal may contain heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into a cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by
25 microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or *in vitro* fertilization, but rather is directed to the introduction of a recombinant DNA molecule. This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA. In the typical transgenic animal, the
30 transgene causes cells to express a recombinant form of the subject

polypeptide. The terms "chimeric animal" or "mosaic animal" are used herein to refer to animals in which the recombinant gene is found, or in which the recombinant is expressed in some but not all cells of the animal. The term "tissue-specific chimeric animal" indicates that the recombinant gene is
5 present and/or expressed in some tissues but not others.

As used herein, the term "transgene" means a nucleic acid sequence (encoding, for example, a human interferon polypeptide, a human monoclonal antibody, and the like) that is partly or entirely heterologous (*i.e.*, foreign, to the transgenic animal or cell into which it is introduced) or is homologous to
10 an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (*e.g.*, it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene according to
15 the present invention will include one or more transcriptional regulatory sequences, polyadenylation signal sequences, and any other nucleic acids, such as introns, that may be necessary for optimal expression of a selected nucleic acid.

The techniques used to isolate and characterize the nucleic acids and
20 proteins of the present invention are well known to those of skill in the art and standard molecular biology and biochemical manuals may be consulted to select suitable protocols without undue experimentation (see, for example, Sambrook *et al.*, "Molecular Cloning: A Laboratory Manual," 2nd ed., 1989, Cold Spring Harbor Press; the contents of which is incorporated herein by
25 reference in its entirety).

Following longstanding law convention, the terms "a" and "an" as used herein, including the claims, mean "one or more."

Abbreviations

30 Abbreviations used in the present specification include the following:

aa, amino acid(s); bp, base pair(s); cDNA, DNA complementary to RNA; nt, nucleotide(s); SSC, sodium chloride-sodium citrate; DMSO, dimethyl sulfoxide.

5 *Chicken ovomucoid gene expression control region nucleic acid sequences*

A series of PCR amplifications of template chicken genomic DNA were used to isolate the gene expression control region of the chicken ovomucoid locus. The region of the chicken genome lying between the 3' end of the ovoinhibitor gene and the 5' transcription start site of the ovomucoid gene was
10 PCR amplified using the primers OVINs 2 (5'-TAGGCAGAGCAATAGGACTCTCAACCTCGT-3')(SEQ ID NO: 1) and OVMUa2 (5'-AAGCTTCTGCAGCACTCTGGGAGTTACTCA-3')(SEQ ID NO: 2), as described in detail in Example 1 below and Fig. 1. The resulting fragment of approximately 10 kb was blunt-ended and cleaved with the
15 restriction endonuclease *Bam* HI, producing two fragments of about 4.7 kb and about 5.5 kb in length. These two fragments were subcloned into the linearized plasmid vector pBluescript KS II (+/-). As described in Example 3 below, each insert was sequenced using the primers SEQ ID NOS: 5 – 25 shown in Figs. 2 and 3. The compiled nucleic acid sequence (SEQ ID NO:
20 26) of the approximately 10 kb nucleic acid region that is 5' upstream of the chicken ovomucoid transcription start site is shown in Fig. 4.

SEQ ID NO: 26 includes the ovoinhibitor gene 3' untranslated region described by Scott *et al.* (1987, *J. Biol. Chem.* 262: 5899 –5909) from base positions 1-255, as shown in Fig. 4. A CR1-like element (Scott *et al.*, 1987,
25 *Biochemistry* 26: 6831-6840; Genbank Accession No: M17966) is located at base positions 2761-3024, as shown in Fig. 4. The region of SEQ ID NO: 26, from base positions 9403-9920 as shown in Fig. 4, has been described in Genbank Accession No: J00897 and in Lai *et al.*, 1979, *Cell* 18: 829-842 and includes a portion of the 5' untranslated region of the ovomucoid gene.

It is contemplated that any nucleic acid sequence encoding a polypeptide may be operably linked to the novel isolated avian ovomucoid gene expression control region so as to be expressed in a transfected cell that, in one embodiment of the present invention, may be an avian cell. For example, a plasmid construct contacting the cloned ovomucoid promoter region and a desired polypeptide-encoding nucleic acid sequence may be transfected into cultured quail or chicken oviduct cells, which may then be incubated to synthesize a polypeptide detectable with antibodies directed against the desired polypeptide.

The novel isolated chicken ovomucoid gene expression control region of the present invention comprises the nucleotide elements that are positioned 5' upstream of the transcription start site of the native chicken ovomucoid locus and necessary for the regulated expression of a downstream polypeptide-encoding nucleic acid. It is contemplated that this region includes those transcription control regions regulatable by hormones including, for example, steroid hormones and the like.

One aspect of the present invention, therefore, provides a novel isolated nucleic acid that comprises the nucleotide sequence SEQ ID NO: 26, shown in Fig. 4 (GenBank Accession No. _____), and derivatives and variants thereof located immediately 5' upstream of the transcription start site of the chicken ovomucoid gene locus.

In one embodiment of the present invention, the isolated nucleic acid may be isolated from an avian selected from the group consisting of a chicken, a turkey, a duck, a goose, a quail, a pheasant, a ratite, an ornamental bird or a feral bird.

In another embodiment of the present invention, the isolated nucleic acid is obtained from a chicken. In this embodiment, the isolated nucleic acid has the sequence of SEQ ID NO: 26, as shown in Fig. 4, or a variant thereof.

Another aspect of the present invention provides nucleic acids that can hybridize under high, medium, or low stringency conditions to an isolated

nucleic acid that encodes a chicken ovomucoid gene expression control region having all, a derivative of, or a portion of the nucleic acid sequence SEQ ID NO: 26, shown in Fig. 4. The nucleotide sequence shown in SEQ ID NO: 26 and determined from the isolation of a chicken ovomucoid gene expression control region allows for the generation of probes useful identifying homologs of ovomucoid gene expression control regions in other species, especially in other avian species.

Fragments of a nucleic acid encoding a portion of the subject ovomucoid gene expression control region are also within the scope of the invention. As used herein, a fragment of the nucleic acid encoding an active portion of an ovomucoid gene expression control region refers to a nucleotide sequence having fewer nucleotides than the nucleotide sequence encoding the entire nucleic acid sequence of the ovomucoid gene expression control region.

In one embodiment of the present invention, the nucleotide sequence of the isolated DNA molecule of the present invention may be used as a probe in nucleic acid hybridization assays for the detection of the ovomucoid gene expression control region. The nucleotide sequence of the present invention may be used in any nucleic acid hybridization assay system known in the art including, but not limited to, Southern blots (Southern, E.M., 1975, *J. Mol. Biol.* 98: 508), Northern blots (Thomas et al., 1980, *Proc. Natl. Acad. Sci.* 77: 5201-05), Colony blots (Grunstein et al., 1975, *Proc. Natl. Acad. Sci.* 72: 3961-65), and the like. Alternately, the isolated DNA molecules of the present invention can be used in a gene amplification detection procedure such as a polymerase chain reaction (Erlich et al., 1991, *Science* 252: 1643-51; the contents of which is incorporated herein by reference in its entirety) or in restriction fragment length polymorphism (RFLP) diagnostic techniques, such as those described by Watson et al. (pps. 519-522 and 545-547 in "Recombinant DNA," 2nd edition, 1992, Scientific American Books; the contents of which is incorporated herein by reference).

Nucleotides constructed in accordance with the present invention can be labeled to provide a signal as a means of detection. For example, radioactive elements such as ^{32}P , ^3H , and ^{35}S or the like provide sufficient half-life to be useful as radioactive labels. Other materials useful for labeling synthetic nucleotides include fluorescent compounds, enzymes, and chemiluminescent moieties. Methods useful in selecting appropriate labels and binding protocols for binding the labels to the synthetic nucleotides are well known to those of skill in the art. Standard immunology manuals such as Promega: Protocol and Applications Guide (2nd Edition, 1991, Promega Corp., Madison, WI; the contents of which is incorporated herein in its entirety) may be consulted to select an appropriate labeling protocol without undue experimentation.

In another embodiment of the present invention, an isolated nucleic acid molecule of the present invention includes a nucleic acid that is at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, still more preferably at least about 95%, and even more preferably at least about 99%, identical to a chicken-derived ovomucoid gene expression control region -encoding nucleic acid molecule as depicted in SEQ ID NO: 26.

In another embodiment of the present invention, an avian ovomucoid gene expression control region gene or nucleic acid molecule can be an allelic variant of SEQ ID NO: 26.

The present invention also contemplates the use of antisense nucleic acid molecules that are designed to be complementary to a coding strand of a nucleic acid (*i.e.*, complementary to an mRNA sequence) or, alternatively, complimentary to a 5' or 3' untranslated region of the mRNA. Another use of synthetic nucleotides is as primers (DNA or RNA) for a polymerase chain reaction (PCR), ligase chain reaction (LCR), or the like.

Synthesized nucleotides can be produced in variable lengths. The number of bases synthesized will depend upon a variety of factors, including

the desired use for the probes or primers. Additionally, sense or anti-sense nucleic acids or oligonucleotides can be chemically synthesized using modified nucleotides to increase the biological stability of the molecule or of the binding complex formed between the anti-sense and sense nucleic acids.

- 5 For example, acridine substituted nucleotides can be synthesized. Protocols for designing isolated nucleotides, nucleotide probes, and/or nucleotide primers are well-known to those of ordinary skill. Additionally, such nucleotides, probes, and primers can be purchased commercially from a variety of sources (e.g., Sigma Genosys, The Woodlands, TX or The Great
10 American Gene Co., Ramona, CA).

- The nucleic acid sequence of a chicken ovomucoid gene expression control region nucleic acid molecule (SEQ ID NO: 26) of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules by procedures such as, but not limited to, insertion into a cell
15 for replication by the cell, by chemical synthesis or by procedures such as PCR or LCR, (b) obtain nucleic acid molecules which include at least a portion of such nucleic acid molecules, including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions and the like, (c) obtain ovomucoid gene expression control region nucleic acid
20 homologs in other species including avian species such as, but not limited to, turkey, duck, goose, quail, pheasant, parrot, finch, ratites including ostrich, emu and cassowary and, (d) to obtain isolated nucleic acids capable of hybridizing to an avian ovomucoid gene expression control region nucleic acid and be used to detect the presence of nucleic acid-related sequences by
25 complementation between the probe and the target nucleic acid.

- Such nucleic acid homologs can be obtained in a variety of ways including by screening appropriate expression libraries with antibodies of the present invention, using traditional cloning techniques to screen appropriate libraries, amplifying appropriate libraries or DNA using oligonucleotide primers
30 of the present invention in a polymerase chain reaction or other amplification

method, and screening public and/or private databases containing genetic sequences using nucleic acid molecules of the present invention to identify targets. Examples of preferred libraries to screen, or from which to amplify nucleic acid molecules, include but are not limited to mammalian BAC
5 libraries, genomic DNA libraries, and cDNA libraries. Similarly, preferred sequence databases useful for screening to identify sequences in other species homologous to chicken ovomucoid gene expression control region include, but are not limited to, GenBank and the mammalian Gene Index database of The Institute of Genomics Research (TIGR).

10

Codon-optimized proteins

Another aspect of the present invention provides a recombinant DNA molecule comprising the novel isolated avian ovomucoid gene expression control region of the present invention operably linked to a selected
15 polypeptide-encoding nucleic acid insert, which may express the nucleic acid insert when transfected to a suitable host cell such as an avian cell. In one embodiment of the present invention, the nucleic acid insert is placed in frame with a signal peptide sequence, whereby translation initiation from the transcript starts with the signal peptide and continues through the nucleic acid
20 insert, thereby producing an expressed polypeptide having the desired amino acid sequence.

It is anticipated that the recombinant DNA may further comprise a polyadenylation signal sequence that will allow the transcript directed by the novel ovomucoid gene expression control region to proceed beyond the
25 nucleic acid insert encoding a polypeptide and allow the transcript to further comprise a 3' untranslated region and a polyadenylated tail. Any functional polyadenylation signal sequence may be linked to the 3' end of the nucleic acid insert including, but not limited to, the SV40 polyadenylation signal sequence, the bovine growth hormone adenylation sequence, or the like, or
30 derivatives thereof.

Another aspect of the present invention is to provide nucleic acid sequences of a protein optimized for expression in avian cells, and derivatives and fragments thereof.

One embodiment of the present invention is a recombinant DNA molecule comprising the isolated avian ovomucoid gene expression control region of the present invention, operably linked to a nucleic acid insert encoding a polypeptide, and a polyadenylation signal sequence optionally operably linked thereto. It is contemplated that when the recombinant DNA is to be delivered to a recipient cell for expression therein. The sequence of the nucleic acid sequence may be modified so that the codons are optimized for the codon usage of the recipient species. For example, if the recombinant DNA is transfected into a recipient chicken cell, the sequence of the expressed nucleic acid insert is optimized for chicken codon usage. This may be determined from the codon usage of at least one, and preferably more than one, protein expressed in a chicken cell. For example, the codon usage may be determined from the nucleic acid sequences encoding the proteins ovalbumin, ovomucoid, lysozyme, ovomucin, ovotransferrin and the like from a chicken.

In one embodiment of the recombinant DNA of the present invention, for example, the nucleic acid insert may encode the human interferon $\alpha 2b$ polypeptide. Optimization of the sequence for codon usage elevates the level of translation in avian eggs. The sequence of the optimized human interferon sequence is disclosed in U.S. Patent Application Serial No: 09/173,864, which is incorporated herein by reference in its entirety.

In yet another embodiment of the present invention, the recombinant DNA comprises the isolated avian ovomucoid gene expression control region operably linked to a nucleic acid encoding a human interferon $\alpha 2b$ and the SV40 polyadenylation sequence.

The protein of the present invention may be produced in purified form by any known conventional technique. For example, chicken cells may be

homogenized and centrifuged and the supernatant then subjected to sequential ammonium sulfate precipitation and heat treatment. The fraction containing the protein of the present invention is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC.

Recombinant nucleic acids, and expression thereof, under the control of an avian ovomucoid promoter

Another potentially useful application of the novel isolated ovomucoid gene expression control region of the present invention is the possibility of increasing the amount of a heterologous protein present in an animal, especially a chicken, by gene transfer. In most instances, a heterologous polypeptide-encoding nucleic acid insert transferred into the recipient animal host will be operably linked with the ovomucoid gene expression control region to allow the cell to initiate and continue production of the genetic product protein. A recombinant DNA molecule of the present invention can be transferred into the extra-chromosomal or genomic DNA of the host.

The recombinant DNA nucleic acid molecules of the present invention can be delivered to cells using conventional recombinant DNA technology. The recombinant DNA molecule may be inserted into a cell to which the recombinant DNA molecule is heterologous (*i.e.* not normally present). Alternatively, as described more fully below, the recombinant DNA molecule may be introduced into cells which normally contain the recombinant DNA molecule, for example, to correct a deficiency in the expression of a polypeptide, or where over-expression of the polypeptide is desired.

For expression in heterologous systems, the heterologous DNA molecule is inserted into the expression system or vector of the present invention in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences, including the novel isolated ovomucoid

gene expression control region.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction
5 enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced to a cell by means of transformation and replicated in cultures, including eukaryotic cells grown in tissue culture.

One aspect of the present invention, therefore, is an expression vector suitable for delivery to a recipient cell for expression of the vector therein. It is
10 contemplated to be within the scope of the present invention for the expression vector to comprise an isolated avian ovomucoid gene expression control region operably linked to a nucleic acid insert encoding a polypeptide and, optionally, a polyadenylation signal sequence. The expression vector of the present invention may further comprise a bacterial plasmid sequence, a
15 viral nucleic acid sequence, and the like, or fragments or variants thereof, that allow for replication of the vector in a suitable host.

The novel isolated avian ovomucoid gene expression control region of the present invention (SEQ ID NO: 26) and a polypeptide-encoding nucleic acid sequence operably linked thereto and, optionally, a polyadenylation
20 signal sequence may be introduced into a virus including, but not limited to, a vaccinia virus, a retrovirus, a poxvirus, and the like. Methods for making a viral recombinant vector useful for expressing a protein under the control of the ovomucoid promoter are analogous to the methods disclosed in U.S. Patent Nos. 4,603,112; 4,769,330; 5,174,993; 5,505,941; 5,338,683;
25 5,494,807; 4,722,848; Paoletti, E., 1996, *Proc. Natl. Acad. Sci.*, 93: 11349-11353; Moss, B., 1996, *Proc. Natl. Acad. Sci.* 93: 11341-11348; Roizman, 1996, *Proc. Natl. Acad. Sci.* 93: 11307-11302; Frolov et al., 1996, *Proc. Natl. Acad. Sci.* 93: 11371-11377; Grunhaus et al., 1993, *Seminars in Virology* 3: 237-252 and U.S. Patent Nos. 5,591,639; 5,589,466; and 5,580,859 relating
30 to DNA expression vectors, *inter alia*; the contents of which are incorporated

herein by reference in their entireties.

Recombinant viruses can also be generated by transfection of plasmids into cells infected with virus. Suitable vectors include, but are not limited to, viral vectors such as lambda vector system λ gt11, λ gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC184, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif.; hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see Studier, F.W. et al., 1990, "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," *Gene Expression Technology*, vol. 185; hereby incorporated herein by reference in its entirety) and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al. (1989, "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Press; the contents of which is incorporated herein by reference in its entirety).

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. The use of eukaryotic recipient host cells permits partial or complete post-translational modification such as, but not only, glycosylation and/or the formation of the relevant inter- or intra-chain disulfide bonds. Host-vector systems include, but are not limited to, the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; vertebrate cell systems infected with virus (e.g., vaccinia virus, adenovirus, retroviruses, and the like); insect cell systems infected with virus (e.g., baculovirus) or avian embryonic cells inoculated with the recombinant nucleic acid. The expression elements of these vectors vary in their strength and specificities. Depending

upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Once the novel isolated ovomucoid gene expression control region of the present invention has been cloned into a vector system, it is ready to be
5 incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, and the like. Alternatively, it is contemplated that the incorporation of the DNA of the present invention into a recipient cell may be
10 by any suitable method such as, but not limited to, viral transfer, electroporation, gene gun insertion, sperm mediated transfer to an ovum, microinjection including pronuclear, nuclear transfer, and the like.

Another aspect of the present invention, therefore, is a method of expressing a heterologous polypeptide in a eukaryotic cell by transfecting the
15 cell with a recombinant DNA comprising an avian ovomucoid gene expression control region operably linked to a nucleic acid insert encoding a polypeptide and, optionally, a polyadenylation signal sequence, and culturing the transfected cell in a medium suitable for expression of the heterologous polypeptide under the control of the avian ovomucoid gene expression control
20 region.

In one embodiment of the method of the present invention, the recipient eukaryotic cell is derived from an avian. In one embodiment, the avian is a chicken.

Yet another aspect of the present invention is a eukaryotic cell
25 transformed with an expression vector according to the present invention and described above. In one embodiment of the present invention, the transformed cell is a chicken oviduct cell and the nucleic acid insert comprises the chicken ovomucoid gene expression control region, a nucleic acid insert encoding a human interferon $\alpha 2d$ and codon optimized for
30 expression in an avian cell, and an SV40 polyadenylation sequence.

It is contemplated that the transfected cell according to the present invention may be transiently transfected, whereby the transfected recombinant DNA or expression vector may not be integrated into the genomic nucleic acid. It is further contemplated that the transfected
5 recombinant DNA or expression vector may be stably integrated into the genomic DNA of the recipient cell, thereby replicating with the cell so that each daughter cell receives a copy of the transfected nucleic acid. It is still further contemplated that the scope of the present invention encompass a transgenic animal producing a heterologous protein expressed from a
10 transfected nucleic acid according to the present invention.

In one embodiment of the present invention, the transgenic animal is an avian selected from a turkey, duck, goose, quail, pheasant, ratite, an ornamental bird or a feral bird. In another embodiment, the present invention contemplates the avian to be a chicken and the heterologous protein
15 produced under the transcriptional control of the isolated avian ovomucoid gene expression control region produced in the white of an egg.

Viral vector cell transformation

An exemplary approach for the *in vivo* introduction of a nucleic acid encoding the novel isolated ovomucoid gene expression control region into a
20 cell is by use of a viral vector containing nucleic acid, e.g., a cDNA, encoding the gene product. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, e.g., by a cDNA
25 contained in the viral vector, are expressed efficiently in cells that have taken up viral vector nucleic acid.

Retrovirus vectors and adeno-associated virus vectors are generally understood to be the recombinant gene delivery system of choice for the transfer of exogenous genes *in vivo*. These vectors provide efficient delivery
30 of genes into cells, and the transferred nucleic acids are stably integrated into

the chromosomal DNA of the host. Recombinant retrovirus can be constructed in the part of the retroviral coding sequence (gag, pol, env) that has been replaced by nucleic acid encoding an ovomucoid gene expression control region, thereby rendering the retrovirus replication defective.

5 Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Ausubel *et al.*, eds, "Current Protocols in Molecular Biology," (1989, Greene Publishing Associates, Sections 9.10-9.14) and other standard laboratory manuals and disclosed in PCT Application No. 99/19472 to Ivarie *et al.*, among others. Examples of
10 suitable retroviruses include pLJ, pZIP, pWE, pEM, avian leucosis virus (ALV), and the like. Examples of suitable packaging virus lines for preparing both ecotropic and amphotropic retroviral systems include psiCrip, psiCre, psi2 and psiAm.

Furthermore, it is possible to limit the infection spectrum of retroviruses
15 and consequently of retroviral-based vectors, by modifying the viral packaging proteins on the surface of the viral particle (see, for example PCT publications WO93/25234, WO94/06920, and WO94/11524). For instance, strategies for the modification of the infection spectrum of retroviral vectors include coupling antibodies specific for cell surface antigens to the viral env protein (Roux *et al.*, 1989, *Proc. Natl. Acad. Sci.* 86: 9079-9083; Julan *et al.*, 1992, *J. Gen. Virol.* 73: 3251-3255 and Goud *et al.*, 1983, *Virology* 163: 251-254) or
20 coupling cell surface ligands to the viral env proteins (Neda *et al.*, 1991, *J. Biol. Chem.* 266: 14143-14146)(all of which are incorporated herein by reference in their entireties). Coupling can be in the form of the chemical
25 cross-linking with a protein or other variety (e.g. lactose to convert the env protein to an asialoglycoprotein), as well as by generating fusion proteins (e.g. single-chain antibody/env fusion proteins). This technique, while useful to limit or otherwise direct the infection to certain tissue types, can also be used to convert an ecotropic vector into an amphotropic vector.

Another viral gene delivery system useful in the present invention utilizes adenovirus-derived vectors. The genome of an adenovirus can be manipulated such that it encodes a gene product of interest, but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle (see, for example, Berkner et al., 1988, *BioTechniques* 6: 616; Rosenfeld et al., 1991, *Science* 252: 43 1434; and Rosenfeld et al., 1992, *Cell* 68: 143-155, all of which are incorporated herein by reference in their entireties). Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. The virus particle is relatively stable and amenable to purification and concentration, and as above, can be modified so as to affect the spectrum of infectivity. Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (e.g., retroviral DNA). Most replication-defective adenoviral vectors currently in use and therefore favored by the present invention are deleted for all or parts of the viral E1 and E3 genes but retain as much as 80% of the adenoviral genetic material (see, e.g., Jones et al., 1979, *Cell* 16:683; Berkner et al., *supra*; and Graham et al., 1991, pp. 109-127 in "Methods in Molecular Biology," vol. 7, E. J. Murray, ed., Humana, Clifton, N.J., all of which are incorporated herein by reference in their entireties. Expression of an inserted gene such as, for example, encoding the human interferon $\alpha 2b$, can be under control of the exogenously added ovomucoid gene expression control region sequences.

Yet another viral vector system useful for delivery of, for example, the subject avian ovomucoid gene expression control region operably linked to a nucleic acid encoding a polypeptide, is the adeno-associated virus (AAV). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AAV

vector, such as that described in Tratschin et al., 1985, *Mol. Cell. Biol.* 5: 3251-3260, can be used to introduce DNA into cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see, for example, Hermonat et al., 1984, *Proc. Natl. Acad. Sci.* 81: 6466-6470; 5 Tratschin et al., 1985, *Mol. Cell. Biol.* 4: 2072-2081; Wondisford et al., 1988, *Mol. Endocrinol.* 2: 32-39; Tratschin et al., 1984, *J. Virol.* 51: 611-619; and Flotte et al., 1993, *J. Biol. Chem.* 268: 3781-3790; all of which are incorporated herein by reference in their entireties).

10 *Non-viral expression vectors*

Most non-viral methods of gene transfer rely on normal mechanisms used by eukaryotic cells for the uptake and intracellular transport of macromolecules. In preferred embodiments, non-viral gene delivery systems of the present invention rely on endocytic pathways for the uptake of the 15 subject ovomucoid gene expression control region and operably linked polypeptide-encoding nucleic acid by the targeted cell. Exemplary gene delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes.

In a representative embodiment, a nucleic acid comprising the novel 20 isolated ovomucoid gene expression control region of the present invention can be entrapped in liposomes bearing positive charges on their surface (e.g., lipofectins) and (optionally) which are tagged with antibodies against cell surface antigens of the target tissue (PCT publication WO91/06309; the content of which is incorporated herein by reference in its entirety).

25 In similar fashion, the gene delivery system comprises an antibody or cell surface ligand that is cross-linked with a gene binding agent such as polylysine (see, for example, PCT publications WO93/04701, WO92/22635, WO92/20316, WO92/19749, and WO92/06180; the contents of which are incorporated herein by reference in their entireties). It will also be appreciated 30 that effective delivery of the subject nucleic acid constructs via receptor-

mediated endocytosis can be improved using agents which enhance escape of gene from the endosomal structures. For instance, whole adenovirus or fusogenic peptides of the influenza HA gene product can be used as part of the delivery system to induce efficient disruption of DNA-containing endosomes (Mulligan et al., 1993, *Science* 260-926; Wagner et al., 1992, *Proc. Natl. Acad. Sci.* 89: 7934; and Christiano et al., 1993, *Proc. Natl. Acad. Sci.* 90: 2122; the contents of which are incorporated herein by reference in their entireties). It is further contemplated that a recombinant DNA molecule comprising the novel isolated ovomucoid gene expression control region of the present invention may be delivered to a recipient host cell by other non-viral methods including by gene gun, microinjection, sperm-mediated transfer, or the like.

Transgenic animals

Another aspect of the present invention concerns transgenic animals, such as chickens, that contain a transgene comprising the novel isolated ovomucoid gene expression control region of the present invention and that preferably (though optionally) express a heterologous gene in one or more cells in the animal. Suitable methods for the generation of transgenic avians having heterologous DNA incorporated therein are described, for example, in PCT Published Application No. WO 99/19472 to Ivarie et al., and incorporated herein by reference in its entirety.

In various embodiments of the present invention, the expression of the transgene may be restricted to specific subsets of cells, tissues or developmental stages utilizing, for example, cis-acting sequences acting on the ovomucoid gene expression control region of the present invention and which control gene expression in the desired pattern. Tissue-specific regulatory sequences and conditional regulatory sequences can be used to control expression of the transgene in certain spatial patterns. Moreover, temporal patterns of expression can be provided by, for example, conditional

recombination systems or prokaryotic transcriptional regulatory sequences.

One embodiment of the present invention, therefore, is a transgenic avian having a heterologous polynucleotide sequence comprising a nucleic acid insert encoding the heterologous polypeptide and operably linked to the novel isolated avian ovomucoid gene expression control region. In an embodiment of the present invention, the transgenic avian is selected from a chicken, a turkey, a duck, a goose, a quail, a pheasant, a ratite, an ornamental bird or a feral bird. In another embodiment of the present invention, the transgenic avian is a chicken.

10 In still another embodiment of the transgenic avian of the present invention, the transgenic avian includes an avian ovomucoid gene expression control region comprising the nucleic acid sequence in SEQ ID NO: 26, or a degenerate variant thereof.

15 In yet another embodiment of the transgenic avian of the present invention, the transgenic avian further comprises a polyadenylation signal sequence.

In still yet another embodiment of the transgenic avian of the present invention, the polyadenylation signal sequence is derived from the SV40 virus.

20 In another embodiment of the transgenic avian of the present invention, the nucleic acid insert encoding a polypeptide has a codon complement optimized for protein expression in an avian.

25 In another embodiment of the transgenic avian of the present invention, the transgenic avian produces the heterologous polypeptide in the serum or in the white of an egg.

In another embodiment of the transgenic avian of the present invention, the transgenic avian produces the heterologous polypeptide in an egg white.

30 The present invention is further illustrated by the following examples,

which are provided by way of illustration and should not be construed as limiting. The contents of all references, published patents and patents cited throughout the present application are hereby incorporated by reference in their entireties.

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Example 1: PCR amplification of Ovomuroid promoter

Sense primer OVINS2, 5'-
TAGGCAGAGCAATAGGACTCTCAACCTCGT-3' (SEQ ID NO: 1) and the
antisense primer, OVMUa2, 5'-
10 AAGCTTCTGCAGCACTCTGGGAGTTACTCA-3' (SEQ ID NO: 2) were
designed according to the sequences of chick ovoinhibitor exon 16 (Genbank
Accession No: M16141) and a fragment of the chick ovomucoid promoter
region (Genbank Accession No: J00897) respectively. The template DNA for
PCR amplification of the ovomucoid promoter region was prepared from
15 blood obtained from a white leghorn chick.

A series of different PCR conditions were carried out to optimize
synthesis of the approximately 10.0 kb product, the results of which are
shown in Fig. 2. In these tests, the template DNA concentrations were 500
ng, 100 ng, 50 ng, or 10 ng. Two sets of primers, OVINS1 (SEQ ID NO: 3)
20 and OVMUa1 (SEQ ID NO: 4), or OVINS2 (SEQ ID NO: 1) and OVMUa2
(SEQ ID NO: 2) shown in Fig. 3, three Mg⁺⁺ concentrations (1.0 mM, 1.5 mM
and 2.0 mM) and annealing temperatures from 50°C to 70°C were used.

The results of the tests were as shown in Fig. 2. As shown in lanes 1
through 8, test reactions using 500 ng DNA template; OVINS1 (SEQ ID NO:
25 3) and OVMUa1 (SEQ ID NO: 4) primers; 60 mM Tris-SO₄, pH9.1; 18 mM
(NH₄)₂SO₄; 1.0 mM Mg²⁺; and annealing temperatures between 50°C to 58°C
gave no specific DNA product. Also, as shown in lanes 17 through 24 of Fig.
2, test reactions using 100 ng DNA template; OVINS1 and OVMUa1 primers;
60 mM Tris-SO₄, pH9.1; 18 mM (NH₄)₂SO₄; 1.0 mM Mg²⁺; and annealing
30 temperatures between 50°C to 58°C did not result in the production of

specific bands. However, as shown in lanes 9 through 16 of Fig. 2, test reactions using 500 ng DNA template; OVINs2 (SEQ ID NO: 1) and OVMUa2 (SEQ ID NO: 2) primers; 60 mM Tris-SO₄, pH9.1; 18 mM (NH₄)₂SO₄; 2 mM Mg²⁺; and annealing temperatures between 60°C to 68°C produce a band of the desired length of approximately 10 kb. As shown in lanes 25 through 32, reaction conditions containing 100 ng DNA template; OVINs2 (SEQ ID NO: 1) and OVMUa2 (SEQ ID NO: 2) primers; 60 mM Tris-SO₄, pH9.1; 18 mM (NH₄)₂SO₄; 2 mM Mg²⁺; and annealing temperatures between about 60°C to about 68°C gave an increased yield of the desired product.

An approximately 10 kb product was, therefore, detected when the following conditions were used: the optimum DNA template concentration was between about 50 ng to 500 ng; the primers were OVINs2 (SEQ ID NO: 1) and OVMUa2 (SEQ ID NO: 2); the Mg²⁺ concentration was 2 mM; and the annealing temperature was at or between about 60°C to about 68°C. Each 50 µl PCR reaction consisted of 50 ng or 100 ng of template DNA, 0.1 µg of each primer, 5 µl buffer B (Elongase Enzyme Mix, Gibco BRL), 1 ml of 10 µM dNTP solution, and distilled deionized water. The PCR protocol comprised one cycle at 94°C for 30 secs; thirty cycles, each at 94°C for 30 secs, 60°C for 30 secs, and 68°C for 10 mins; one cycle at 68°C for 10 mins than 35°C for 30 mins; with a final hold at 4°C. Resulting PCR products were examined using 0.65% agarose gel analysis.

Example 2: Cloning of PCR products.

The PCR products were purified by standard methods. Briefly, phenol: chloroform: isoamyl alcohol (24:25:1) extraction and chloroform extraction were performed, followed by precipitation of the DNA by adding 3M sodium acetate, pH 5.2, at a final concentration of 0.3M together with 2.5 volumes of 100% ethanol. The DNA pellet was dried and dissolved in deionized water and then sequenced on a ABI3700 automatic sequencer (Applied Biosystems) using the primers OVINs2 (SEQ ID NO: 1) and OVMUa2 (SEQ

ID NO: 2) to confirm the identity of each PCR product. After the identities were confirmed, the approximately 10 kb PCR product was treated with T4 polynucleotide kinase to add a phosphate to the 5' end. Mung bean nuclease was used to remove overhanging adenines from the ends of the PCR products, thereby producing a blunt end. The PCR product was purified by phenol: chloroform: isoamyl alcohol extraction and chloroform extraction and precipitated as described above. The 10 kb product was then cleaved using *Bam* HI to give two fragments of about 4.7 and about 5.5 kb, respectively.

The vector plasmid pBluescript II KS (+/-) was cut by *Bam* HI and *Eco* RV and treated with calf intestinal alkaline phosphatase. The DNA fragments to be ligated into the vector were analyzed by agarose gel electrophoresis and purified from agarose gel slices using a NucleoTrap Nucleic Acid Purification Kit (Clontech). The 4.7 kb and 5.5 kb fragments were inserted into the *Bam* HI/*Eco* RV-treated pBluescript to give the pBS-OVMUP4.7 and pBS-OVMUP5.5 constructs, respectively.

Positive clones were screened by *Xba* I/*Xho* I digestion. Clone pBS-OVMUP4.7 gave fragments of about 4.7 kb and 2.96 kb. Clone pBS-OVMUP5.5 gave fragments of about 5.5 kb and 2.96 kb. The presence of the 4.7 kb in putatively positive clones was further confirmed using *Xba* I/*Hind* III digestion, giving three fragments of 0.5 kb, 4.2 kb, and 2.9 kb. Putative positive clones with an insert of about 5.5 kb insert were further confirmed by *Xba* I/*Kpn* I digestion, giving three fragments of 2 kb, 3.5 kb and 2.96 kb.

A construct containing the entire 10 kb PCR product cloned into the pBluescript KS II (+/-) vector was made by taking a 4.7 kb *Bam* HI/*Xho* I fragment from the pBS-OVMUP4.7 plasmid and inserting it into the *Bam* HI/*Xba* I cleaved sites of pBS-OVMUP5.5. The *Xho* I and *Xba* I cut ends were blunt-ended by treating the digested fragments with Klenow enzyme and dNTPs at 25°C for 15 mins before the digestion with *Bam* HI.

Example 3: Sequencing

Plasmids pBS-OVMUP4.7 and pBS-OVMUP5.5 were sequenced from both ends of each insert as shown in Fig. 1. The initial primers were T7 and
5 T3 having the nucleic acid sequences 5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO: 5) and 5'-ATTAACCCTCACTAAAGGGA-3' (SEQ ID NO: 6), respectively. Subsequent primers (SEQ ID NOS: 7 - 25), as shown in Fig. 3, were designed according to the sequence results as they became available. The approximately 10 kb sequence was edited and assembled using
10 ContigExpress software of the Vector NTI Suite, version 6.0 (InforMax, Inc.). The region of the approximately 10 kb PCR product, described in Example 1 above, encompassing the *Bam* HI junction was sequenced using OVMUa9 (SEQ ID NO 27) and OVINs9 (SEQ ID NO 28) primers (shown in Fig. 3).

Each sequence chromatogram was visually checked for sequence
15 accuracy and to locate base ambiguities. Regions containing ambiguous bases were re-sequenced with the same primer or, if still ambiguous, with a new primer designed to sequence the complementary strand. Sequencing of the original 10 kb PCR fragment using the primers OVMUa9 (SEQ ID NO 27) and OVINs9 (SEQ ID NO 28) showed that the subcloned inserts of the
20 plasmids pBS-OVMUP4.7 and pBS-OVMUP5.5 included all of the nucleic acid sequence of the parent fragment. No intervening *Bam* HI – *Bam* HI fragments were included in the final sequence SEQ ID NO: 26. SEQ ID NO: 26, shown in Fig. 4, is the sequence of the region lying between the 3' end of the ovoinhibitor gene and the transcription start site of the ovomucoid-
25 encoding region.

Example 4: Expression in Transfected Cultured Avian Oviduct Cells of Human Interferon $\alpha 2b$ Regulated by the 12kb Ovomucoid Promoter

The oviduct of a Japanese quail (*Coturnix coturnix japonica*) will be removed and the magnum portion minced and enzymatically dissociated with
5 0.8 mg/ml collagenase (Sigma Chemical Co., St. Louis, MO) and 1.0 mg/ml dispase (Roche Molecular Biochemicals, Indianapolis, IN) by shaking and titrating for 30 minutes at 37°C. The cell suspension will then filtered through sterile surgical gauze, washed three times with F-12 medium (Life Technologies, Grand Island, NY) by centrifugation at 200 x g, and
10 resuspended in OPTIMEM™ (Life Technologies) such that the OD₆₀₀ will be approximately 2. 300 µl of the cell suspension will be plated in each well of a 24-well dish. For each transfection, 2.5 µl of DMRIE-C liposomes (Life Technologies) and 1 µg of DNA, comprising the ovomucoid promoter region (SEQ ID NO: 26) and a chicken optimized human interferon α -2b encoding
15 sequence (as disclosed in U.S. Patent Application Serial No: 09/173,864, incorporated herein by reference in its entirety, will be preincubated for 15 minutes at room temperature in 100 µl of OPTIMEM™, and then added to the oviduct cells. Cells with DNA/liposomes will be incubated for about 5 hours at 37°C in 5% CO₂. Next, 0.75 ml of DMEM (Life Technologies), supplemented
20 with 15% fetal bovine serum (FBS) (Atlanta Biologicals, Atlanta, GA), 2X penicillin/streptomycin (Life Technologies), 10⁻⁶ M insulin (Sigma), 10⁻⁸ M α -estradiol (Sigma), and 10⁻⁷ M corticosterone (Sigma) will be added to each well, and incubation continued for about 72 hours. Medium will then be harvested and centrifuged at 110 x g for 5 minutes. The supernatant will be
25 analyzed by ELISA for human interferon $\alpha 2b$ content.

The human interferon $\alpha 2b$ contents of medium derived from cultured oviduct cells transfected with a plasmid having human interferon $\alpha 2b$ cDNA operably linked to the chicken ovomucoid promoter region (SEQ ID NO: 26) will be determined.